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Agouti-Signaling Protein Impacts Blastocyst Development In Cattle

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Introduction

Bovine *In Vitro* Fertilization (IVF)

- Despite advancements in oocyte and embryo culture systems, only 20-40% of presumptive zygotes will reach the blastocyst stage *in vitro* [1,2].
- A limiting factor to IVF is lack of knowledge of the mRNAs and proteins necessary for oocyte maturation and early embryonic development [3,4].

Agouti signaling protein (*ASIP*):

- 132 amino acid secreted protein
- Highly abundant in the bovine oocyte (RNA-Sequencing data, Yao lab; **Fig. 1**).
- The function of ASIP in the ovary is unknown.
- A role in lipid metabolism has been established in other bovine tissues including adipose [5] and mammary epithelial cells [6].

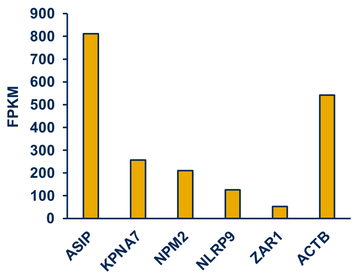


Figure 1. *ASIP* expression in the bovine oocyte in relation to other highly expressed oocyte genes.

Objectives:

1. Characterize the effect of recombinant ASIP (rASIP) supplementation during *in vitro* maturation (IVM).
2. Determine the impact of ASIP ablation on early embryonic development.

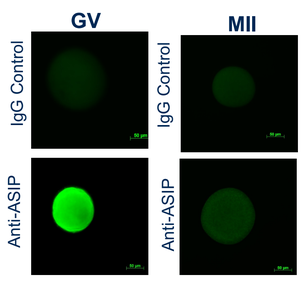


Figure 2. Immunofluorescent localization using anti-bovine ASIP 1˚ antibody (10 ug/mL) of ASIP in immature (GV) and mature (MII) bovine oocytes.

Materials and Methods

Experiment 1:

In vitro embryo production

- Cumulus-oocyte complexes (COCs) were cultured in IVM (IVF Bioscience) containing either 0 (control), 1, 10, 100, 500, or 1000 ng/mL of rASIP (R&D Systems) for 22-24 h.
- Mature (MII) oocytes were collected and the remaining COCs were fertilized.
- Presumptive zygotes were placed in IVC (IVF Bioscience) and blastocyst development was assessed on Day 8.

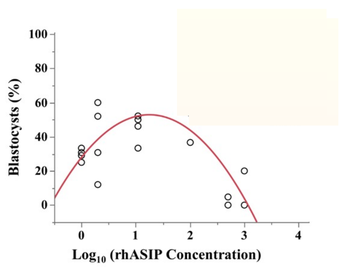


Figure 3. Results of preliminary experiment to determine optimal rASIP concentration for blastocyst rate.

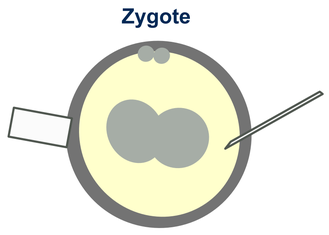
Nile Red Staining

- MII oocytes or blastocysts were stained using Nile Red (Sigma-Aldrich cat. #72485) according to previously published methods [5].

Experiment 2:

Zygote Microinjection

- Presumptive zygotes (12-18 h post-insemination) were microinjected with approximately 20 pl of siRNA targeting bovine *ASIP*.
- Uninjected and negative siRNA injected zygotes served as controls.
- 4 cell embryos (4 pools 10) were collected to validate ASIP knockdown via RT-qPCR according to previously published methods [6].
- Blastocyst development was assessed on Day 8.



12-18 h post fertilization

Results

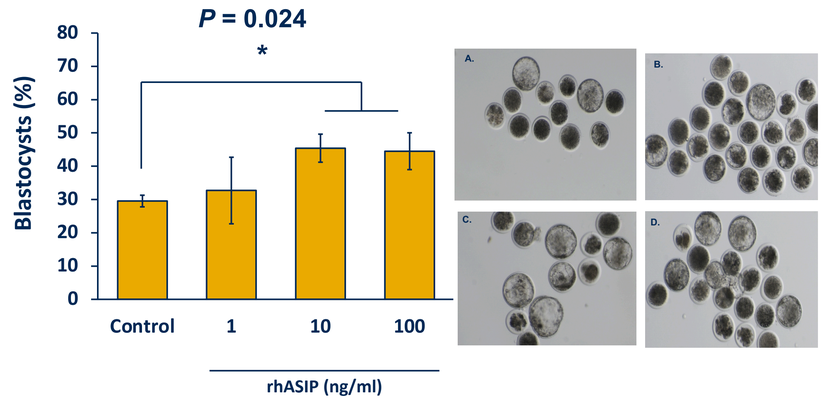


Figure 4. ASIP Supplementation During IVM Improves Blastocyst Development
Addition of 10 and100 ng/mL of rASIP significantly increased blastocyst development (n = 4 reps of 30-3 COCs/treatment) as revealed by Dunnett's test comparing treatment groups to the control. Representative images of D8 blastocysts following treatment of either A) Control B) 1 ng/mL C) 10 ng/mL and D) 100 ng/mL

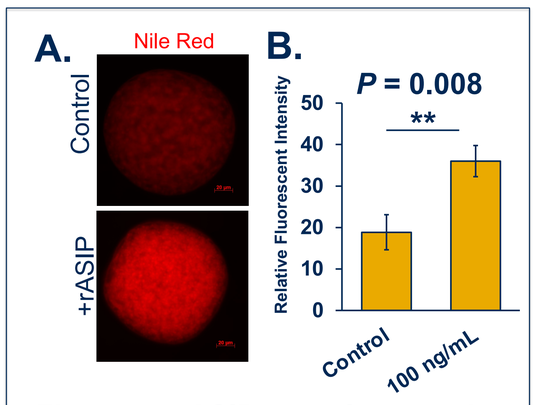


Figure 5. ASIP supplementation during IVM increases oocyte lipid content.
(A) Representative images of MII oocytes stained with Nile Red to localize lipids following supplementation with 0 or 100 ng/mL ASIP during IVM. (B) ASIP supplementation significantly increased lipid content of mature oocytes (n = 8-10 oocytes/treatment; Student's t-Test).

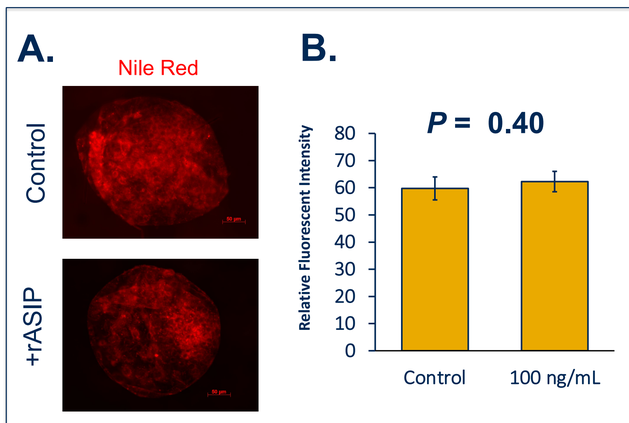


Figure 6. ASIP supplementation during IVM does not alter blastocyst lipid content. (A) Representative images of Day 8 blastocysts stained with Nile Red. (B) Blastocyst lipid content was not affected by ASIP supplementation during IVM (n = 8-10 embryos/treatment; Student's t-Test).

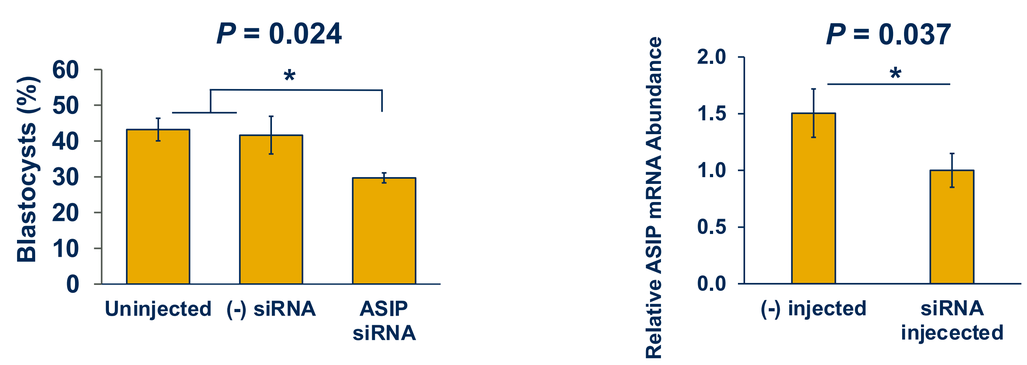


Figure 7. (A) Statistical analysis using a contrast revealed blastocyst development was significantly decreased by 13% in embryos injected with ASIP siRNA (29% ± 2.98) in comparison to uninjected and negative siRNA injected zygotes (n = 5 replications of 30-38 zygotes/treatment). (B) The ASIP siRNA species injected into zygotes successfully decreased ASIP abundance in 4-cell embryos (n = 4 pools of 10/treatment; Student's t-Test) at determined by RT-qPCR.

Conclusions

- **Exogenous ASIP supplementation during IVM:**
  - **Increases the rate of blastocyst development**
  - **Increases oocyte but not embryo lipid content**
- **siRNA mediated ASIP knockdown at the zygote stage deceased blastocyst development by 13%.**
- **Future work will characterize the function of ASIP during oocyte maturation and early embryonic development in cattle.**

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SARE Northeast Sustainable Agriculture Research and Education

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